Phylogenetic analysis of Micracidini bark beetles (Coleoptera: Curculionidae) demonstrates a single trans-Atlantic disjunction and inclusion of *Cactopinus* in the New World clade

Bjarte H. Jordal,[1] Johanna Kaidel

Abstract—Micracidini (Coleoptera: Curculionidae: Scolytinae) is an unusual tribe of mainly bigynous bark beetles found in dry forests and scrublands in Afrotropical and Neotropical regions. Their phylogenetic relationship to other bark beetle groups is poorly known with few clues from external morphology. Hence, a phylogenetic analysis of five genes (COI, EF-1a, 28S, CAD, ArgK) and morphological (internal and external) data was conducted to test potential sister group relationships, including 56 outgroup genera in 22 tribes, and 18 species in 10 genera of Micracidini. *Cactopinus* Schwarz – a genus with many cactus feeding species – was nested within a clade of all Neotropical and Nearctic genera. The New World was colonised by an Afrotropical ancestor about 75–85 million years ago, where cactus feeding in *Cactopinus* evolved much later. All analyses indicated a paraphyletic clade of Afrotropical micracidines, strongly supporting inclusion of the Ipini genus *Dendrochilus* Schedl in *Afromicracis* Schedl. Hypoborini appear to be one of the more plausible sistergroup candidates to Micracidini, and revealed morphological similarity in protibial and proventricular characters. Most phylogenetic results were supported independently by morphological and molecular data and therefore document the power of thorough examination of morphological characters analysed properly in a phylogenetic context.

Introduction

Bark beetles in the tribe Micracidini (Coleoptera: Curculionidae: Scolytinae) are found in the Afrotropical, Nearctic, and Neotropical regions (Table 1). This is one of few tribes in Scolytinae that possibly exhibits a disjunct trans-Atlantic distribution – a geographical split which dates back to the Paleocene or longer (Jordal and Cognato 2012). The Afrotropical taxa include the six genera *Afromicracis* Schedl, *Lanurgus* Eggers, *Phloeocurus* Wood, *Pseudomicracis* Eggers, *Saurotocis* Wood, and *Traglostus* Schedl; two of these (*Pseudomicracis* and *Saurotocis*) are mainly found in Madagascar. New World genera are more numerous and include *Hylocurus* Eichhoff, *Micracis* LeConte, *Micracisella* Blackman, *Phloeocleptus* Wood, *Pseudothysanoes* Blackman, *Stenoclyptus* Blackman, *Stevewoodia* Bright, and *Thysanoes* LeConte. The tribe is morphologically fairly homogeneous, with relatively few traits characterising the group (Fig. 1). However, most females bear a distinct tuft of long setae on a short antennal scape (Fig. 1C, see Wood 1982), and, irrespective of sex, have a distinctive apical plate of the proventriculus (Fig. 2, see also Lopez-Buenfil *et al.* 2001). Other traits are apparently similar to species in Dryocoetini, Ipini, and Cactopinini (Wood 1978, 1986), but a detailed phylogenetic analysis is needed to test such relationships.

Species of Micracidini differ biologically in several ways from other bark beetles. The large majority of species are bigamous where exactly two females join the male in a shared tunnel under bark (Wood 2007). Females are therefore the courting sex having the most extravagant and species diagnostic features (Kirkendall *et al.* 2014). Very little is known about the Afrotropical

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B.H. Jordal,[1] J. Kaidel, Department of Natural History, University Museum, University of Bergen, P.O. Box 7800, NO-5020 Bergen, Norway

[1]Corresponding author (e-mail: bjarte.jordal@uib.no).
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Table 1. Current classification of Micracidini and putatively close relatives in Cactopinini, Ipini, and Corthylini.

Tribe	Genus	Distribution
Corthylini	*Mimiocurus* Schedl, 1957	Africa
Ipini	*Dendrochilus* Schedl, 1959	Africa
Micracidini	*Afromicracis* Schedl, 1959	Africa
	Lanurgus Eggers, 1920	Africa and Madagascar
	Traglostus Schedl, 1938	Africa
	Phloeocurus Wood, 1984	Africa
	Pseudomicracis Eggers, 1920	Madagascar (and Africa)
	Saurotocis Wood, 1984	Madagascar
	Micracis LeConte, 1868	North and South America
	Micracisella Blackman, 1928	North America
	Hylocurus Eichhoff, 1872	North and South America
	Phloeocleptus Wood, 1956	North and South America
	Stenoclyptus Blackman, 1943	North America
	Thysanoes LeConte, 1876	North and South America
	Pseudothysanoes Blackman, 1920	North and South America
	Stevewoodia Bright, 2010	Central America (Caribbean)
Cactopinini	*Cactopinus* Schwarz, 1899	North America

Fig. 1. Lateral view of (A) *Cactopinus rhois*, (B) *Phloeocleptus cristatus*, (C) *Lanurgus* species, (D) *Afromicracis* species, (E) *Dendrochilus arundinarius*, and (F) *Mimiocurus setifer*.

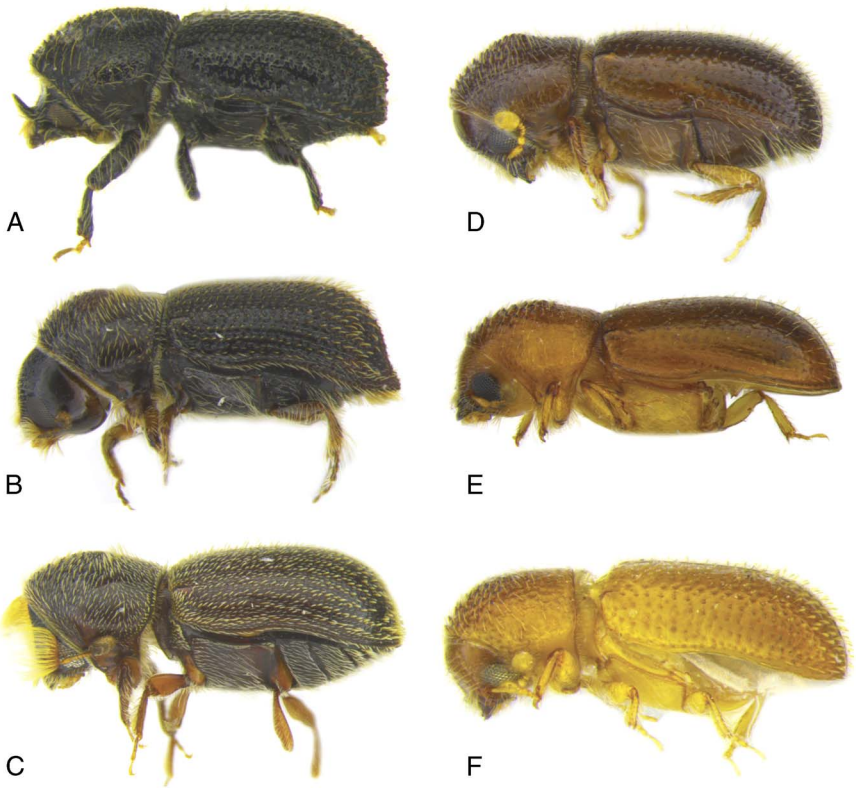
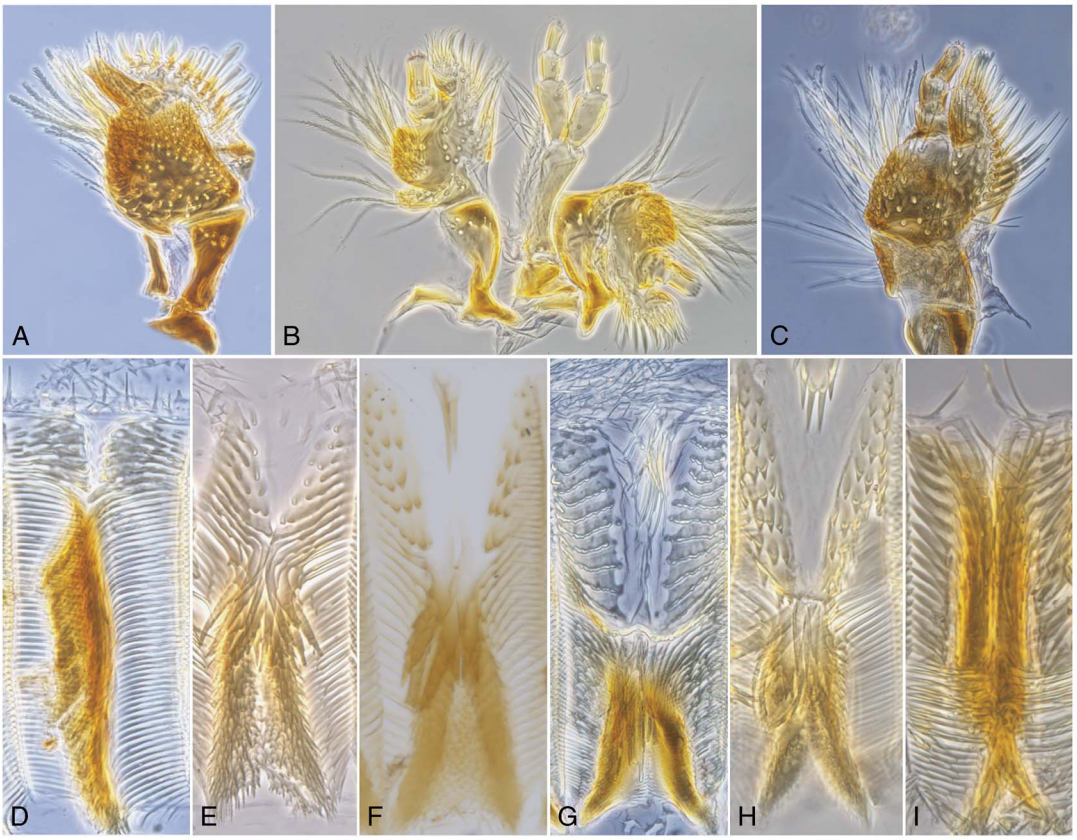


Fig. 2. Maxillae (A–C) and proventriculus (D–I) of species in Micracidini and *Cactopinus*. Internal face of left maxilla in (A) *Cactopinus* species, (B) *Phloeocleptus cristatus*, and (C) *Lanurgus xylographus*. The inner face of one proventriculus blade in (D) *Cactopinus* species, (E) *Cactopinus rhois*, (F) *Phloeocleptus cristatus*, (G) *Lanurgus xylographus*, (H) *Afromicracis* species, and (I) *Micracisella nanula*.

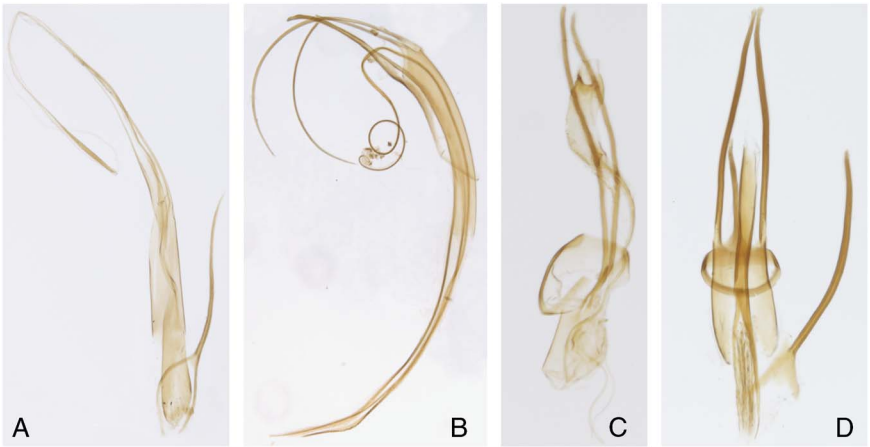


genera, but field observations indicate that they are fairly similar in behaviour to their Neotropical relatives (B.H.J., unpublished data). A large proportion of species on both continents are found breeding in dry and old twigs or branches and constitute an important part of the wood-boring fauna in arid scrublands and dry, deciduous forests. Typical species breed in inner bark, but the tribe as a whole is found in a broad range of woody tissues. Some species of *Lanurgus* tunnel into the sapwood as do many *Hylocurus*, or they tunnel through the pith of twigs such as in *Micracisella* (see Wood 1982). Others are found in lianas or small shrubs, particularly so in the Afrotropical *Afromicracis* and *Lanurgus*.

Monophyly of Micracidini has rarely been disputed since Wood's (1986) revision of Scolytinae genera. The tiny species of *Afromicracis* was one of the later additions to the tribe and this genus contains several species with little sexual dimorphism that is otherwise typical for micracidine species. In that respect the genus shows several similarities with the rare Afrotropical genus *Dendrochilus* Schedl, currently placed in Ipini (Wood 1986). Both genera are fairly uncharacteristic and could be placed several places in the classification based on gross external morphology (Fig. 1). Molecular data has nevertheless placed *Afromicracis* with confidence in Micracidini, close to *Lanurgus*, and there is little reason to suggest other relationships for this genus (Jordal and Cognato 2012). The question remains, however, where *Dendrochilus* belongs in this scheme.

Another taxon that is possibly closely related to Micracidini is the enigmatic genus *Cactopinus* Schwarz. All species in this genus are found in dry

Fig. 3. Male genitalia of (A) *Cactopinus rhois*, (B) *Phloeocleptus cristatus*, (C) *Afromicracis* species, (D) and *Micracisella nanula*. The spiculum gastrale is present in all species but detached in photographs B and C.



scrublands, known from Mexico to the southwestern United States of America. In these dry terrains they have adapted to feed on novel host plants, especially cactus (Cactaceae) and *Agave* Linnaeus (Asparagaceae), but there are also several species feeding and breeding in *Pinus* Linnaeus (Pinaceae) and *Rhus* Linnaeus (Anacardiaceae) (Atkinson 2010). A separate subfamily was originally erected by Chamberlin (1939) for this genus based on the unique presence of a pair of horns arising from the lower frons (Atkinson 2010). Some authors believe the genus is most closely related to Micracidini, either near *Phloeocleptus* and *Stenoclyptus* (Wood 1957), or more distantly related (Blackman 1943; Wood 1986), while others suggest a totally separate position of *Cactopinus* in its own subfamily (Bright 2014). Recent molecular phylogenetic analyses have suggested a potentially close relationship to Micracidini, in particular the Neotropical and Nearctic genera of the tribe (Jordal *et al*. 2008; Jordal and Cognato 2012).

This study provides the first thorough phylogenetic analysis of Micracidini genera and close relatives, based on DNA sequences from five gene fragments, and morphological characters. We are testing several taxonomic and biogeographical hypotheses related to the current classification. First we test the monophyly of Micracidini and thereby explore likely sister group candidates for the tribe, with particular focus on *Cactopinus*. Second, we test the hypothesis that all Afrotropical and New World genera are reciprocally monophyletic, which would be contrary to Wood's (1986) hypothesis on mixed clades of genera from different continents. A third hypothesis involves the monophyly of the Afrotropical genera, with a special focus on *Afromicracis* and its relationship to *Dendrochilus*.

Central to our test scheme is the information provided from morphological characters, particularly internal or otherwise hidden anatomical characters. We give particular attention to characters coded from mouth parts, the posterior margin of the head, sutures on the thorax, hind wings, male genitalia, and the inner face of the eight proventricular blades (Figs. 2–3). Character states coded from these body parts have rarely been used in phylogenetic analyses of bark and ambrosia beetles, with a few notable exceptions (Jordal *et al*. 2002b; Jordal and Hewitt 2004). Internal anatomical characters have furthermore shown promise in associating species to tribe or higher clades (Jordal *et al*. 2002a; Jordal 2009, 2010, 2012) and are applicable to the phylogenetic analyses of many bark beetle groups. We therefore test the hypothesis that internal morphology is diagnostic for well-supported nodes in molecular phylogenetic trees.

Materials and methods

We included 80 species of Scolytinae in the phylogenetic analyses (Table 2). Two weevil

Table 2. List of samples and their geographical origin, their DNA voucher number, and GenBank accession numbers.

Tribe	Species	Country	Locality	Collector	Voucher	CO1	EF-1a	28S	CAD	ArgK
Entiminae										
Polydrusiini	*Polydrusus cervinus*	Norway	Gvarv, Telemark	B. Jordal	EnPol01	HQ883653	HQ883729	HQ883568	HQ883793	HQ883884
Conoderinae										
Menemachini	*Homoemetamelus* species	Uganda	Kibale	B. Jordal	CsXxA01	HQ883643	HQ883723	HQ883558	HQ883785	HQ883872
Scolytinae										
Bothrosternini	*Cesinus lecontei*	Costa Rica	Birri	L. Kirkendall	BoCne01	JX263780	AF308397	AF308352	JX263985	JX263876
Bothrosternini	*Eupagiocerus dentipes*	Costa Rica	Birri	L. Kirkendall	BoEup01	JX263781	JX264074	JX263670	JX263986	JX263877
Cactopinini	*Cactopinus nasutus*	Mexico	Coxcatlan	T. Atkinson	CaCac03	–	–	KX099968	–	KX099984
Cactopinini	*Cactopinus rhois*	United States of America	California, Riverside, San Bernadino	A. Cognato	CaCac01	JX263783	JX264075	EU090343	–	JX263878
Cactopinini	*Cactopinus* species 2	Mexico	Coxcatlan	T. Atkinson	CaCac02	–	KX100019	KX099967	KX099992	KX099983
Corthylini	*Corthylus rubricollis*	Costa Rica		L. Kirkendall	CoCor01	JX263786	JX264078	JX263678	JX263996	JX263885
Corthylini	*Dendroterus defectus*	Panama		L. Kirkendall	CoDen01	JX263787	JX264079	JX263679	JX263997	JX263886
Corthylini	*Mimiocurus setifer*	Tanzania	Uluguru Mountain	V. Grebennikov	IpDen02	KX100007	KX100021	KX099970	KX099994	KX099986
Corthylini	*Pityophtorus micrographus*	Norway	Trondheim	B. Jordal	CoPit01	EU191840	EU191872	JX263682	JX264001	JX263889
Cryphalini	*Cosmoderes* species	Papua New Guinea	Asiki Road, Bulolo	B. Jordal	CrPti01	–	JX264095	JX263698	JX264015	JX263906
Cryphalini	*Cryphalus longus*	Russia	Primorsky, Andreev	B. Jordal	CrCry04	JX263796	JX264088	JX263689	JX264008	JX263898
Cryphalini	*Ernoporicus spessivtzevi*	Russia	Primorsky, Anisimovka	B. Jordal	CrErn04	JX263800	JX264091	JX263694	JX264011	JX263901
Cryphalini	*Procryphalus mucronatus*	United States of America	Utah, Alta Canyon	B. Jordal	CrPro01	JX263804	JX264094	JX263697	JX264014	JX263905
Crypturgini	*Aphanarthrum capense*	South Africa	Eastern Cape, Ecca Pass	B. Jordal	CgAph02	EU143701	EU143705	JX263672	JX263988	JX263879
Crypturgini	*Crypturgus borealis*	Canada		B. Jordal	CgCry02	AF187130	AY500991	JX263675	JX263991	JX263882
Diamerini	*Diamerus inermis*	Tanzania	Udzungwa National Park	B. Jordal	DiDia03	JX263808	JX264101	JX263702	JX264020	JX263909
Diamerini	*Strombophorus spathulatus*	Tanzania	Udzungwa National Park	B. Jordal	DiStr04	JX263810	JX264103	JX263704	JX264022	JX263911
Dryocoetini	*Cyrtogenius africus*	South Africa	Eastern Cape, Ecca Pass	B. Jordal	DrCyr01	JX263811	JX264104	JX263705	JX264023	JX263912
Dryocoetini	*Dryocoetes autographus*	Russia	St. Petersburg	M. Mandelshtam	DrDry01	JX263816	JX264109	HQ883565	HQ883791	HQ883880
Dryocoetini	*Ozopemon uniseriatus*	Papua New Guinea	Asiki Road, Bulolo	B. Jordal	DrOzo02	AF438506	AF439740	JX263714	JX264031	JX263921
Dryocoetini	*Thamnurgus cylindricus*	Cameroon	Mount Cameroon	B. Jordal	DrCyr03	JX263813	JX264106	JX263707	JX264025	JX263914
Hexacolini	*Gymnochilus reitteri*	Costa Rica	Castilla	L. Kirkendall	CtGym01	HQ883644	–	EU090353	HQ883786	HQ883873
Hexacolini	*Scolytodes acuminatus*	Costa Rica		L. Kirkendall	CtSct01	EU191844	EU191876	EU090351	HQ883790	HQ883877
Hylastini	*Hylastes opacus*	Sweden	Gotland	B. Jordal	HtHyt05_1	HQ883660	HQ883732	HQ883927	HQ883799	JX263937
Hylastini	*Hylurgops glabratus*	Norway	Øksenøy, Bindalen	B. Jordal	HtHyg02	JX263830	JX264119	JX263728	JX264045	JX263936

Table 2. *Continued*

Tribe	Species	Country	Locality	Collector	Voucher	CO1	EF-1a	28S	CAD	ArgK
Hylesinini	*Ficicis despectus*	Papua New Guinea	Madang, Beitata	B. Jordal	HlFic02	AY376999	AY377063	JX263719	JX264036	JX263928
Hylesinini	*Hapalogenius oblongus*	Uganda	Kibale Forest, Fort Portal	B. Jordal	HlHap02	JX263823	AF308412	JX263721	JX264038	JX263930
Hylesinini	*Hylastinus fankhauseri*	Austria	Vienna	A. Petrov	HlHlt02	JX263824	JX264114	JX263722	JX264039	JX263931
Hylesinini	*Hylesinopsis fasciatus*	Cameroon	Mount Cameroon	B. Jordal	HlHnp01	JX263825	JX264115	JX263723	JX264040	JX263932
Hylesinini	*Hylesinus fraxini*	Sweden	Gotland, E. Visby	B. Jordal	HlHyl02	HQ883657	AF308409	AF308365	HQ883796	HQ883887
Hylurgini	*Dendroctonus micans*	Estonia	Rohukula, Haapsalu	K. Voolma	ToDen01	HQ883680	HQ883749	HQ883591	HQ883824	JX263969
Hylurgini	*Hylurgopinus rufipes*	Canada	Manitoba, Winnipeg		ToHrg01	JX263864	JX264145	JX263768	JX264068	JX263974
Hylurgini	*Tomicus piniperda*	Norway	Lom, Elveseter	B. Jordal	ToTom01	HQ883681	HQ883750	HQ883592	HQ883825	HQ883911
Hypoborini	*Hypoborus ficus*	Portugal	Madeira, Porto santo	B. Jordal	HyHyb01	AY377006	AY377070	EU090350	–	JX263939
Ipini	*Acanthotomicus* species 1	Cameroon	Bomana falls	B. Jordal	IpAca01	JX263833	JX264121	JX263731	JX264048	JX263940
Ipini	*Dendrochilus arundinarius*	Tanzania	Udzungwa National Park	B. Jordal	IpDen01	KX100006	KX100020	KX099969	KX099993	KX099985
Ipini	*Ips duplicatus*	Estonia	Mooste, Põlva	K. Voolma	IpIps03	JX263834	JX264122	JX263733	JX264050	JX263942
Ipini	*Pityogenes bistridentatus*	Ukraine	Crimea, Yalta	M. Mandelshtam	IpPit01	HQ883662	HQ883734	HQ883573	HQ883801	HQ883891
Micracidini	*Afromicracis congonus*	Cameroon	Limbe, Ekonjo	B. Jordal	MiMio02	JX263839	–	JX263738	JX264053	JX263945
Micracidini	*Afromicracis* species I	South Africa	Western Cape, Gouna	B. Jordal	MiMio12	KX100012	KX100025	KX099976	KX100000	–
Micracidini	*Afromicracis* species D	Tanzania	Udzungwa National Park	B. Jordal	MiMio09	KX100011	KX100024	KX099975	KX099999	–
Micracidini	*Afromicracis* species H	Cameroon	Mount Cameroon	B. Jordal	MiMio03	JX263840	–	JX263739	JX264054	JX263946
Micracidini	*Hylocurus femineus*	United States of America	Arizona, Madera Canyon	B. Jordal	MiHyl01	AF187108	AF186678	JX263736	JX264052	–
Micracidini	*Hylocurus longstoni*	United States of America	Texas, Weslake Hills	B. Jordal	MiHyl02	KX100008	KX100022	KX099971	KX099995	–
Micracidini	*Lanurgus* species K	South Africa	Western Cape, Nature's Valley	B. Jordal	MiLan02	JX263837	JX264125	KX099972	KX099996	KX099987
Micracidini	*Lanurgus* species O	Tanzania	Udzungwa National Park	B. Jordal	MiLan14	KX100009	–	KX099973	KX099997	KX099988
Micracidini	*Micracis carinulatus*	United States of America	Arizona, Herb Martyr	B. Jordal	MiMic01	AF187107	AF186677	AF375303	–	–
Micracidini	*Micracis swainei*	United States of America	Texas, Travis, Austin	T. Atkinson	MiMic02	KX100010	KX100023	KX099974	KX099998	–
Micracidini	*Micracisella nanula*	United States of America	Texas, Woodville	T. Atkinson	MiMis01	KX100013	KX100026	KX099977	KX100001	–
Micracidini	*Phloeocleptus cristatus*	Mexico	Michoacan, Uruapan	T. Atkinson	MiPhl01	KX100014	KX100027	KX099978	KX100002	KX099989

Table 2. *Continued*

Tribe	Species	Country	Locality	Collector	Voucher	CO1	EF-1a	28S	CAD	ArgK
Micracidini	*Pseudomicracis* species A	Madagascar	Ranomafana National Park	B. Jordal	MiPsd02	KX100015	–	KX099979	KX100003	KX099990
Micracidini	*Pseudomicracis* species E	Madagascar	Ranomafana National Park	B. Jordal	MiPsd06_1	KX100016	KX100028	KX099980	KX100004	–
Micracidini	*Pseudothysanoes leechi*	United States of America	California, Hastings Reserve	L. Kirkendall	MiPst01	JX263841	JX264126	JX263740	–	–
Micracidini	*Pseudothysanoes yuccae*	Mexico	Palmar del Bravo	T. Atkinson	MiPst03	KX100017	KX100029	KX099981	–	KX099991
Micracidini	*Thysanoes fimbricornis*	United States of America	Maryland, Bowie	T. Atkinson	MiThy01	KX100018	KX100030	KX099982	KX100005	–
Micracidini	New genus	Madagascar		B. Fischer	CrCh_04	JX263793	JX264086	JX263686	–	JX263895
Phloeosinini	*Chramesus asperatus*	United States of America	Arizona, southern Tucson	B. Jordal	PhChr01	JX263843	AF308406	AF308362	JX264056	JX263947
Phloeosinini	*Pseudochramesus acuteclavatus*	Argentina	Salta	B. Jordal	PhPch01	AF375328	AF308404	AF308360	HQ883807	HQ883897
Phloeotribini	*Phloeotribus scarabaeoides*	Spain	Andalusia	M. Kolarik	PtPht05	EU191863	EU191895	JX263759	JX264064	JX263962
Phrixosomini	*Phrixosoma minor*	Costa Rica		L. Kirkendall	PxPrx01	JX263858	JX264140	JX263761	–	JX263964
Phrixosomini	*Phrixosoma uniseriatum*	Cameroon	Limbe	B. Jordal	PxPrx02	JX263859	JX264141	JX263762	JX264065	JX263965
Polygraphini	*Carphoborus perrisi*	Morocco	Agadir	B. Jordal	PoCar01	EU191857	EU191889	JX263748	JX264058	JX263952
Polygraphini	*Chortastus medius*	Cameroon	Limbe	B. Jordal	PoCho01	JX263848	JX264131	JX263750	JX264059	JX263954
Polygraphini	*Dolurgocleptes punctifer*	Madagascar		B. Fischer	DrDol01	EU191846	EU191878	JX263710	JX264027	JX263916
Premnobiini	*Premnobius* species	Sierra Leone	Tiwai Island	B. Jordal	PrPre01	JX263855	JX264137	–	JX264063	JX263960
Scolytini	*Camptocerus aenipennis*	Guyana	Iwokrama	A. Cognato	ScCam02	HQ883676	HQ883745	HQ883587	HQ883818	HQ883907
Scolytini	*Cnemonyx vismiaecolens*	Guyana	Iwokrama	A. Cognato	ScCne01	EU191865	EU191897	HQ883588	HQ883819	HQ883908
Scolytini	*Scolytus intricatus*	Sweden	Oskarshamn	B. Jordal	ScScl02	HQ883677	HQ883746	HQ883589	HQ883820	HQ883909
Scolytini	*Scolytus scolytus*	Denmark	Tofte Skov	J. Pedersen	ScScl06	HQ883678	HQ883747	HQ883590	HQ883821	HQ883910
Scolytoplatypodini	*Remansus sahondrae*	Madagascar	Ranomafana National Park	B. Jordal	SpScp13	KF758331	KF758347	KF758303	KF758319	–
Scolytoplatypodini	*Scolytoplatypus africanus*	Uganda	Kibale	B. Jordal	SpScp01	EU191866	EU191898	AF308391	HQ883822	–
Xyleborini	*Xyleborus affinis*	Uganda	Kibale Forest, Fort Portal	B. Jordal	XyXyl00	AF187138	AF186688	GU808581	GU808621	GU808659
Xyloctonini	*Ctonoxylon flavescens*	Uganda	Masindi	B. Jordal	XcCto03	AY376998	AY377062	JX263775	JX264071	JX263979
Xyloctonini	*Glostatus* species 1	Cameroon	Limbe, Bonadikombe	B. Jordal	XcCry01	JX263868	JX264148	JX263773	–	JX263978
Xyloctonini	*Glostatus* species 2	Tanzania	Udzungwa National Park	B. Jordal	XcCry02	JX263869	JX264149	JX263774	JX264070	–
Xyloctonini	*Scolytomimus phillipinensis*	Papua New Guinea	Madang, Beitata	B. Jordal	XcScm01	JX263871	JX264150	JX263777	–	JX263981
Xyloctonini	*Xyloctonus maculatus*	South Africa	Western Cape, Nature's Valley	B. Jordal	XcXyc01	JX263872	JX264151	JX263778	–	JX263982
Xyloterini	*Trypodendron domesticum*	Norway	Trondheim	B. Jordal	XtTry02	JX263874	JX264152	JX263779	JX264072	JX263983
Xyloterini	*Xyloterinus politus*	United States of America	New Hampshire, Mount Monadnock	B. Jordal	XtXyl01	AF187133	AF186683	AF308395	HQ883838	HQ883924

species were selected as outgroups for rooting of the trees. Micracidini was represented by 18 species from nine genera. Selection of other scolytine tribes was based on previous studies indicating a potentially close relationship of Micracidini and *Cactopinus*, but with otherwise uncertain relationships to other tribes (Jordal *et al*. 2008; Jordal and Cognato 2012). Three species of *Cactopinus* were included as well as representatives from 21 of the currently 26 recognised tribes. DNA was extracted from whole specimens or from the abdomen if only singletons were available. Voucher specimens were based on siblings in collected broods, and the extracted remains of the body.

Nucleotide sequences were obtained by polymerase chain reaction and sequencing using the same protocols as published elsewhere (Jordal *et al*. 2011; Jordal and Cognato 2012). A molecular data matrix was constructed from 3492 nucleotides, including 690 COI, 857 EF1a, 459 CAD and 801 ArgK nucleotides, and 685 aligned positions of the large ribosomal subunit 28S remaining after G-block pruning (Castresana 2000). The complete 28S matrix included 1047 positions when aligned with the software Muscle using default settings (Edgar 2004); G-block settings allowed small final blocks, gap positions, and less strict flanking positions.

Morphological characters were selected based on low variability across multiple genera, with states scored categorically (Appendix 1). A broadest possible representation of characters was aimed for, including adult characters from the head, pronotum, thoracic sclerites, elytra and hind wings, abdomen, legs, proventriculus, and male genitalia; and larval characters. Fewer taxa were available for scoring larval features and the phylogeny was therefore analysed with these characters excluded or included. Internal anatomical characters were observed from dissected beetles treated in 8% potassium hydroxide, washed in water and embedded in Euparal on slides. Terminology for external adult characters follows standard Coleoptera terms (Leschen and Beutel 2014), with additional modifications for internal characters based on Schedl (1931, mouthparts), Nobuchi (1969, proventriculus), Kukalova-Peck and Lawrence (1993, hind wings), and Jordal (1998, male genitalia; 2009, hind wings). Terminology for larval features follow Lekander (1968).

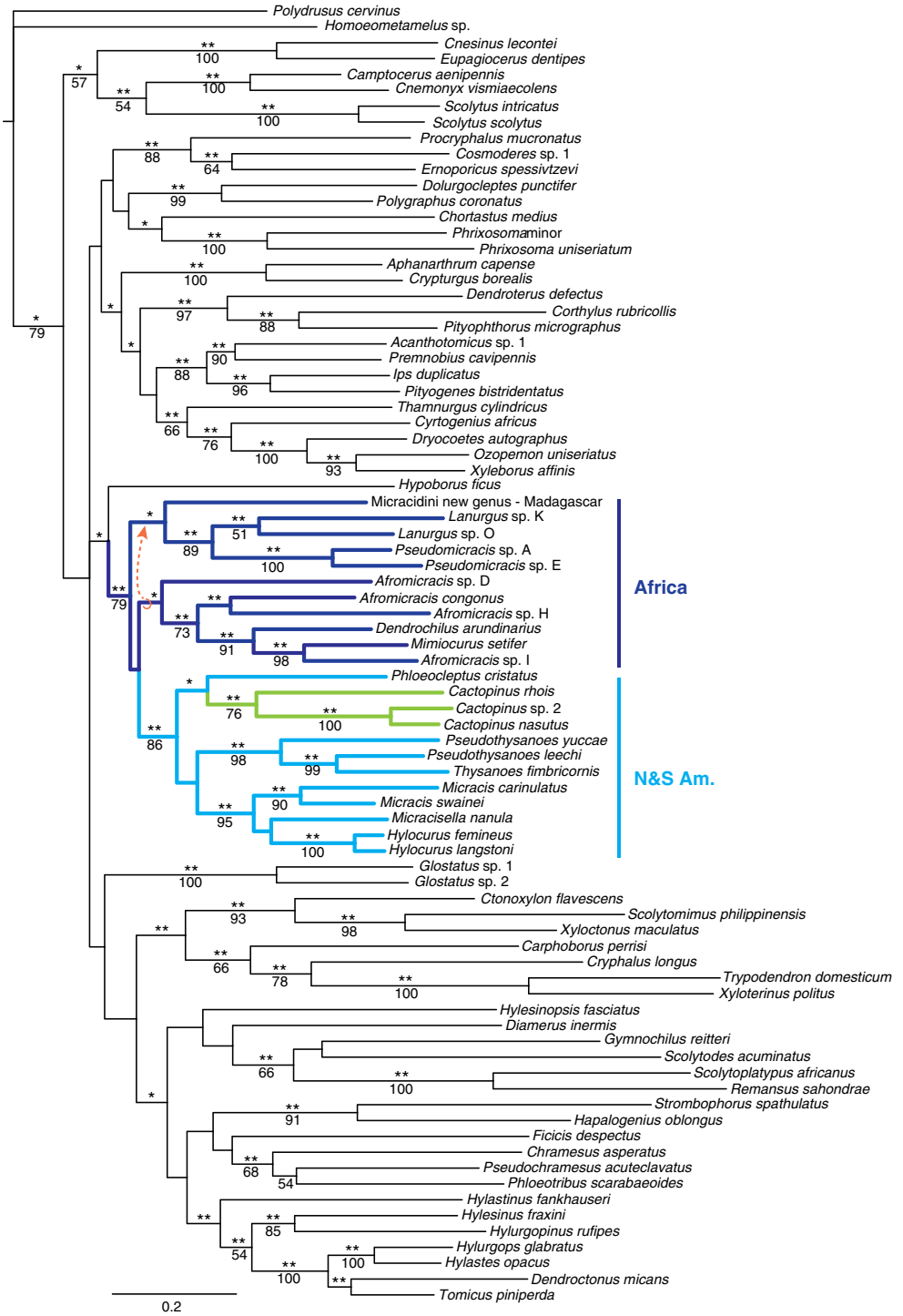
Phylogenetic trees were reconstructed based on Bayesian inference using the software MrBayes 3.2 (Ronquist and Huelsenbeck 2003). Data were divided into eight partitions by genome and nucleotide position, 28S, and morphology (Jordal and Cognato 2012). The best model for each molecular partition was identified using the software MrModeltest (Nylander 2004) in PAUP* (Swofford 2002). Morphological data were analysed using a model with γ distributed rate variation. The data were furthermore analysed by maximum parsimony, which is based on fewest possible assumptions about character evolution (identical rates). Morphological characters were evaluated by its rescaled consistency index over the molecular and morphology-based tree topologies. Incongruence between morphological and molecular data was assessed by the incongruence length difference test (Farris *et al*. 1995) using 100 random addition replicates, with "maxtrees" set to 1000.

Time of divergence was estimated in the software Beast (Drummond and Rambaut 2007) using the same model as for MrBayes. The input file was generated in the Beast module "Beauti" using recommended priors (https://code.google.com/p/beast-mcmc/wiki/ParameterPriors). Twenty million generations were generated, with the first 20% discarded (burn-in) as suggested by stable posteriors and likelihood values evaluated in the software Tracer (Rambaut *et al*. 2014). Molecular rates were calibrated with a relatively precise fossil age for the oldest Scolytinae and other Curculionidae. Because the oldest known Curculionidae fossil (tribe Anthonomini) is 116 million years old (Santos *et al*. 2011), the split between the older Entiminae and the ingroup was set to that age, with a broad standard deviation placing 95% of the node age between 130 and 100 million years old (minimum age of Scolytinae; see Cognato and Grimaldi 2009; Kirejtshuk *et al*. 2009).

Results

Bayesian analysis of all data resulted in topological stability with a potential scale reduction factor of 1.0 and a standard deviation of split frequencies 0.01 (Fig. 4). The parsimony analysis resulted in two trees of length 23 187 steps. The basal resolution in Scolytinae was generally weakly resolved. *Hypoborus* Erichson formed the sister group to Micracidini, albeit moderately

Fig. 4. Tree topology resulting from Bayesian analysis of the combined molecular and morphological data (eight data partitions). Thick branches indicate Micracidini and nested taxa. Neotropical lineages in light blue, Afrotropical in dark blue (including *Dendrochilus* and *Mimiocurus*), *Cactopinus* in green. Posterior probabilities above nodes (* > 0.95; **1.0), bootstrap support values below. Arrow indicates the position of the *Afromicracis* clade in the parsimony analysis (two trees, length = 23 187). N&S Am., North and South America.



supported (posterior probability = 0.95, bootstrap < 50). Micracidini was furthermore paraphyletic with respect to *Cactopinus* and *Dendrochilus* (including the synonymous *Mimiocurus setifer* (Schedl)) and formed a strongly supported clade (posterior probability = 1, bootstrap = 79).

All New World taxa of Micracidini and *Cactopinus* were monophyletic and highly supported (posterior probability = 1, bootstrap = 86). In the Bayesian analysis the Afrotropical taxa formed a two-step grade with a clade consisting of *Afromicracis* and *Dendrochilus* as a weakly supported sister group to the New World clade. All Afrotropical taxa were monophyletic (bootstrap < 50) in the parsimony analysis. Among the micracidine genera with multiple species included, *Afromicracis* was paraphyletic with respect to *Dendrochilus* (posterior probability = 1, bootstrap = 91), and *Pseudothysanoes* with respect to *Thysanoes* (posterior probability = 1, bootstrap = 99).

The Beast analysis of the molecular data was generally congruent with the Bayesian analysis of the same data, showing highly supported clades of Micracidini (posterior probability = 1) and a nested New World clade that includes *Cactopinus* as sister to *Phloecleptus*. The minimum age of Micracidini was estimated to 89.8 million years ago (74.9–106.9), the New World clade 74.8 million years ago (61.8–88.0), and the split between *Phloeocleptus* and *Cactopinus* 64.0 million years ago (51.9–75.6).

Morphology versus molecules

Separate analyses of the morphological and molecular data sets supported a clade of all micracidine genera, which also included *Cactopinus* and *Dendrochilus* (Figs. 5–6). Many other clades were nearly identical between the two data sets, including clades consisting of: Scolytini and Bothrosternini; *Pseudochramesus* Blackman, *Chramesus* LeConte, and *Phloeotribus* Latreille; Hylastini and several genera of Hylurgini; *Cryphalus* Erichson and Xyloterini; Corthylini, Ipini (including *Premnobius* Eichhoff), Dryocoetini and Xyleborini. Less congruent clades were generally not well supported. The incongruence length difference test for morphological versus the molecular data was not significant ($P = 0.39$).

The fit between morphological characters and tree topology was generally high with an average consistency index of 0.36 and a rescaled consistency index of 0.25 as fitted to the molecular topology (Table 3). The highest average rescaled consistency index values were measured for characters on immatures (mainly head capsule features), adult mouthparts, and legs, while a much lower fit to the tree was measured for characters associated with the elytra and flight wings. Many of the nodes increased their support values with the addition of morphological data, particularly in the parsimony analyses (31 increased, two decreased). Among the nodes with synergistic increase in node support was the one connecting all Micracidini (and *Cactopinus*), the node subtending its sister group *Hypoborus*, and nine internal nodes in Micracidini (Figs. 4, 6).

Potential synapomorphies for Micracidini were not present unless *Dendrochilus* was included in the tribe, a genus that is essentially identical to *Afromicracis* in all diagnostic features (Table 4). Micracidini and *Dendrochilus* share a proventricular crop with a bundle of spines just anterior to the apical plate, and, with a few exceptions, containing exactly two setae on the stigmal patch on the flight wings. Many more characters supported a clade containing the above mentioned taxa and *Cactopinus*, for example, a short female scapus (sometimes also in males), a long maxillary palpomere 3, a row of interlocking nodules on the basal inner flange of the elytra (also in some Hylesinini and Hylurgini), an exposed tergite VIII in females (also in Ipini and Dryocoetini/Xyleborini), very long apophyses and manubrium in the male genitalia, and a longitudinally divided and broadly separated apical plate of the proventriculus where each half contain irregularly placed, sharp teeth (less so in cactus feeding species of *Cactopinus*). The latter character state also supported a putative sister relationship to *Hypoborus*, together with the peculiar placement of the most apical denticles on protibiae being displaced posteriorly mesially making a transverse apical row of denticles (or just a single denticle) and with a strong and laterally curved inner apical spine (mucro).

Discussion

Cactopinus is part of Micracidini

Our data clearly demonstrate that *Cactopinus* is a member of Micracidini due to its nested and well-supported position in that tribe. This was not

Fig. 5. Most likely tree topology resulting from the Bayesian analysis of morphological data, based on γ distributed rate variation. This topology is identical to one of the 2146 most parsimonious trees (length = 557 steps, 11 tree islands). All parsimony trees contained a monophyletic clade consisting of Micracidini, *Cactopinus*, *Mimiocurus*, and *Dendrochilus*. Colour and node support as in Figure 4.

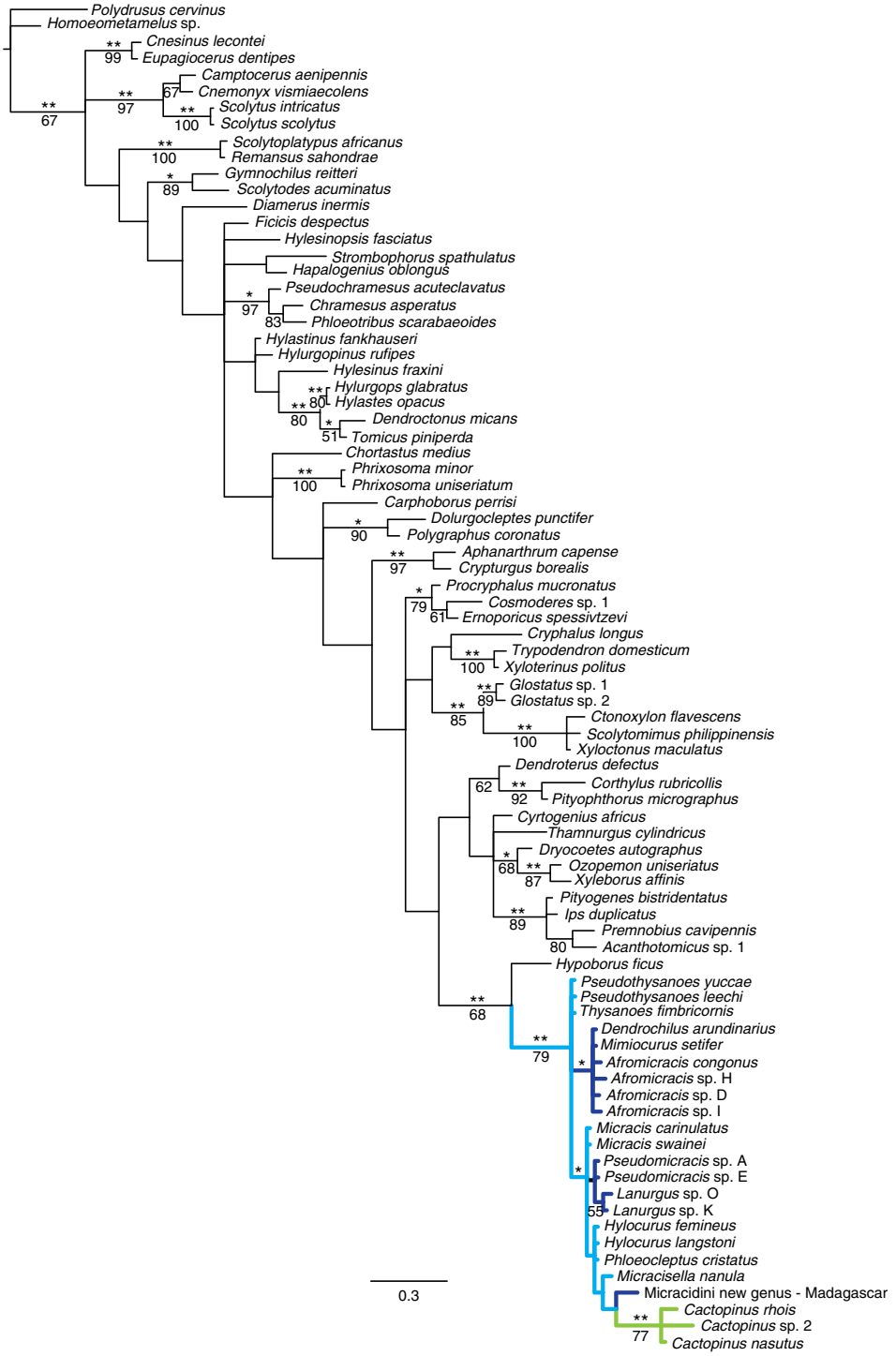


Fig. 6. Tree topology resulting from Bayesian analysis of the molecular data (five genes), based on seven data partitions (codon position per genome, and 28S). The parsimony analysis resulted in identical tree topology (length = 22 541 steps). Colours and node support as in Figure 4. N&S Am., North and South America.

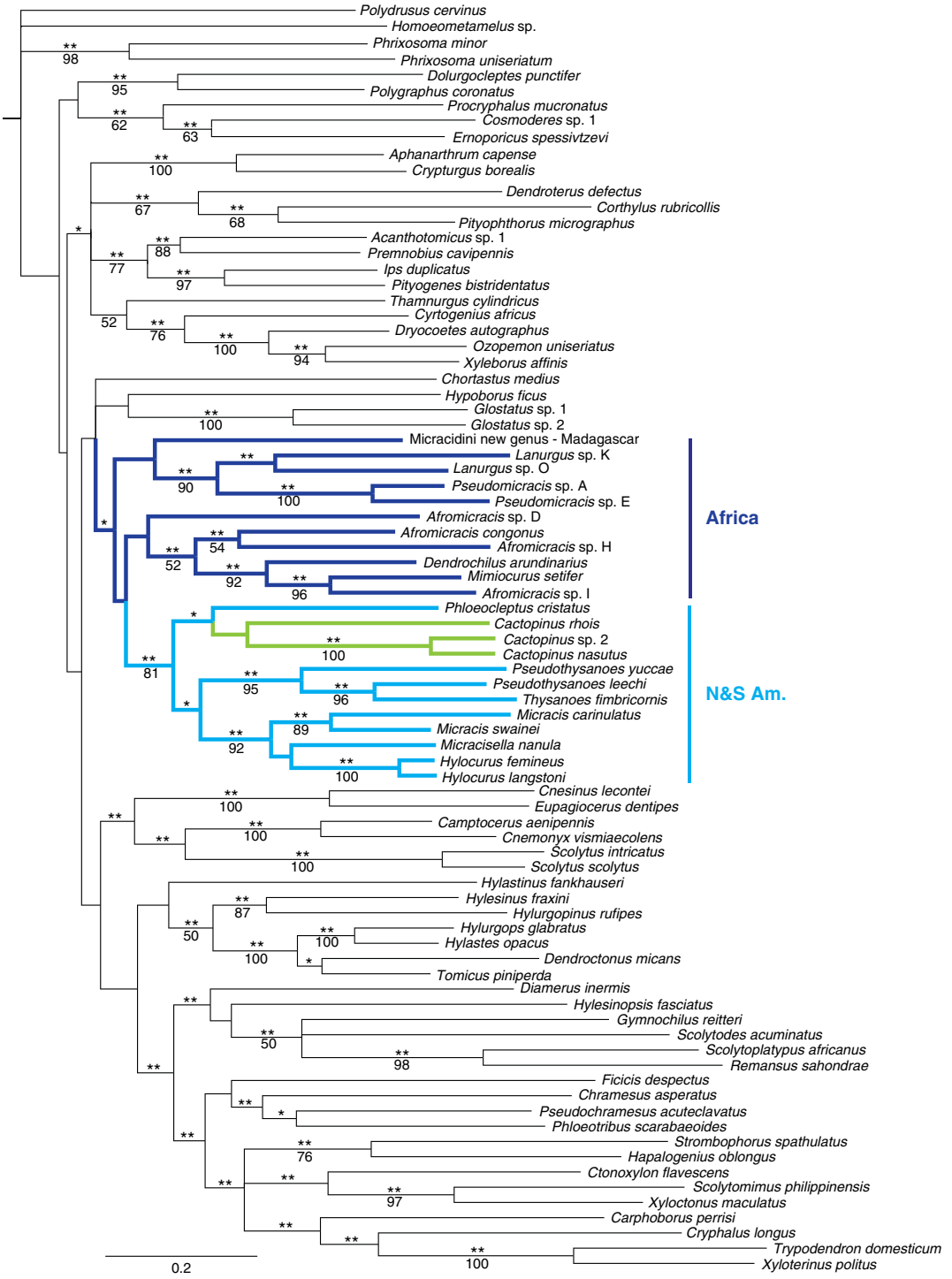


Table 3. The fit of morphological characters on the molecular or morphology-based topologies, as measured by the rescaled consistency index (rci).

Body section	rci molecules	rci morphology
Head	0.30	0.33
Internal (mouth)	**0.32**	**0.37**
External	0.29	0.30
Prothorax	0.26	**0.39**
Mesothorax and metathorax	0.24	0.31
Abdomen	0.26	0.26
Elytra	0.15	0.16
Flight wings	0.10	0.10
Legs	**0.30**	0.32
Proventriculus	0.20	0.23
Male genitalia	0.22	0.23
Immature stages	**0.38**	**0.39**
All characters	0.25	0.28

Note: Characters are grouped by body sections, showing the three highest values in bold.

entirely unexpected based on previous molecular analyses (Jordal *et al*. 2008; Jordal and Cognato 2012), but previous studies were considerably less decisive in terms of node support and revealed ambiguity about the monophyly of the tribe. We have here added two additional species of *Cactopinus* together with several more genera of Micracidini and combine molecular with morphological data. A close relationship between *Cactopinus* and the Micracidini is therefore corroborated and clearly reject alternative hypotheses that these two groups are only distantly related (Wood 1986; Bright 2014).

Cactopinus beetles have a unique appearance, which have led various authors to erect a separate tribe or subfamily for the genus (Blackman 1928, 1943; Chamberlin 1939; Wood 1986; Wood and Bright 1992; Bright 2014). The most striking difference from other bark beetles involves a pair of horns arising from the epistoma of males (Atkinson 2010). In Micracidini the females are usually the ornamented sex, particularly so in the frons and antennae, reflecting their courting behaviour. Micracidine species are generally bigynous, where the male initiate the gallery system and is joined by two successively courting females (Kirkendall *et al*. 2014). The observation that males are the most ornamented in *Cactopinus* (Atkinson 2010) may indicate reversed sex roles, with males courting, although no such observation has been made to date. Evolutionary changes in mating system is nevertheless a fairly labile process in scolytines and reversed sexual dimorphism is therefore not regarded as particularly indicative of relationships (Kirkendall *et al*. 2014). In fact, several lineages in Micracidini show modifications in these traits, including *Micracisella* – the only monogynous genus in Micracidini – and in many *Afromicracis* where the two sexes are unmodified and very similar.

Paired horns are highly derived traits and autapomorphies of this kind provide no evidence for relationships to other genera lacking this trait. The relatively few other distinctive characters in *Cactopinus* intergrade with various micracidine genera, including a rough keel-shaped posterior part of the pronotum (*e.g.*, less developed in some *Cactopinus* and present in some *Lanurgus*), the lack of setae on the stigmal patch of the flight wings (*e.g.*, absent in an undescribed genus from Madagascar, occasionally present as one seta in some micracidines), and smaller dispersed crop spines in the proventriculus (as in *Micracisella*). We furthermore note that the very special male genitalia of *Cactopinus* are similar to those in *Phloeocleptus* – the sister group to *Cactopinus* in our analyses and occasionally placed close to *Cactopinus* in the classification (Wood 1957). The apical plate of the proventriculus is furthermore modified for cactus feeding, and a species such as *Cactopinus rhois* Blackman that feed on *Rhus* has a proventriculus more similar to other micracidines (see Fig. 2E). Overall, there is little reason to argue that *Cactopinus* deserves a separate status as tribe or subfamily (contrary to Bright 2014).

Cactopinus is one of the early offshoots from the North-American branch of Micracidini, which diverged at least some 60 million years ago, in accordance with previous studies based on the same molecular data (Jordal and Cognato 2012). Many micracidine species have habitat preferences similar to *Cactopinus*, which are found predominantly in dry forest types and scrub landscapes (Wood 1982; Atkinson 2010). Even though the climate must have changed repeatedly over evolutionary time, the adaptation to dry forest types seems consistent in a phylogenetic perspective, with only a minority of species found today in moist or wet conditions in temperate or tropical rainforests.

Table 4. Characters supporting phylogenetic relationships in Micracidini and close relatives, with the rescaled consistency index calculated for each character over the entire data matrix, including outgroups.

#	Character	Clade	Exceptions	rci
6	Female scapus short	Micracidini, *Cactopinus*, *Dendrochilus*	Also in males of *Cactopinus*	1.00
13	Maxillary palpomere 3 long	*Cactopinus*, Micracidini (part), *Dendrochilus*	Present in some Dryocoetini	0.22
29	Denticles at protibial apex transversely set	*Hypoborus*, *Dendrochilus*, Micracidini, *Cactopinus*	Not in two of the three *Cactopinus* species	0.48
31	Inner mucro enforced and curved laterally	*Hypoborus*, Micracidini, *Dendrochilus*	Less so in genus *Cactopinus*	0.47
52	Elytra with inner row of locking nodules close to base	Micracidini, *Dendrochilus*, *Cactopinus*	Also in some Hylesinini and Hylurgini	0.21
53	Tergite VIII in females exposed	Micracidini, *Dendrochilus*, *Cactopinus*	Also in Ipini, Dryocoetini, and Xyleborini	0.14
61	Flight wing stigma with two setae	Micracidini (part), *Dendrochilus*	Absent in two Micracidini and all *Cactopinus*	0.12
64	Aedeagal apophyses very long	Micracidini, *Dendrochilus, Cactopinus*	Short in some *Lanurgus* and a new genus	0.35
68	Manubrium very long	Micracidini, *Dendrochilus, Cactopinus*	Short in some *Lanurgus* and a new genus; long, tube-like in *Cactopinus*	0.13
72	Median suture of proventriculus wide open	Micracidini, *Dendrochilus*, *Cactopinus*	Also in many other groups, less developed in two *Cactopinus* species	0.13
74	Apical plate of proventriculus with irregular sharp teeth	*Hypoborus*, *Dendrochilus*, Micracidini, *Cactopinus*	Cactus feeding *Cactopinus* have different apical teeth and plate	0.19
76	Bundle of spines in crop	Micracidini, *Dendrochilus*	Smaller dispersed spines in *Cactopinus, Micracisella*, and a new genus	0.20
84	Larval head capsule with transparent area	Micracidini	*Dendrochilus* and *Cactopinus* larvae not available	1.00

Note: See Supplementary material for a complete list of characters.

It is therefore particularly interesting to find another dry adapted group of beetles as a possible sister group to Micracidini and *Cactopinus*; *Hypoborus* and other genera in the tribe Hypoborini (*e.g.*, *Liparthrum* Wollaston) are all found in similar dry forests of the Mediterranean type. More data are needed to confirm this relationship but we note that this result is consistent with recent phylogenetic analyses of Scolytinae genera (Jordal and Cognato 2012).

Dendrochilus

The Afrotropical "mystery" genus *Dendrochilus* is clearly not a member of Ipini where it is currently placed (Wood 1986). All genetic and morphological data supported a nested position of *D. arundinarius* Schedl within *Afromicracis* and therefore require transfer to this genus. We have examined the type specimens of seven of the nine known species of *Dendrochilus* and the morphological variation is indeed very limited across these species. *Dendrochilus arundinarius* is therefore fully representative for the genus, showing strong affinities with the majority of *Afromicracis* species characterised by obscure antennal sutures and sparse setae on the antennal scapus. As in many micracidine genera they have only few lateral socketed teeth on the protibiae, with the last apical tooth placed mesial to the lateral edge, and the inner apical spine is somewhat enlarged and laterally curved. This feature enables reliable distinction from the Ipini genus *Acanthotomicus* Blandford. Furthermore, the manubrium of the male genitalia is long and pointed (as long as the penis), a feature otherwise rarely observed in Scolytinae. It is therefore not surprising that the phylogenetic analyses of morphological characters also supported a nested position of *Dendrochilus* within *Afromicracis* as observed for the nucleotide data (see Figs. 4–6).

It is interesting to observe that the phylogenetic placement of *M. setifer* is close to *D. arundinarius*, requiring transfer to *Afromicracis* (see Figs. 4–6). This species was originally described in *Mimips* – a synonym of *Acanthotomicus* – and was later placed in the genus *Mimiocurus* Schedl (see Wood and Bright 1992). It would therefore be advisable to revise other species in the genus *Mimiocurus* and perhaps *Acanthotomicus* to potentially discover further synonyms of *Afromicracis*.

The genus *Afromicracis* appears to be one of the oldest genera of Scolytinae, estimated to more than 70 million years old (64–93) based on our data. This is a much older age than other micracidine genera – especially the New World genera are much younger. The Nearctic and Mesoamerican fauna has been more intensively studied compared with the Afrotropical fauna and it is therefore quite possible that a thorough morphological and genetic study of *Afromicracis* will reveal morphological disparity and hence the existence of more than one genus. Until such work has been made (B.H.J., work in progress), we can only conclude that the nine species currently placed in *Dendrochilus* are micracidines and that *M. setifer* is related to these species. Very little is known about the natural history of *Dendrochilus* species, but the few published records on these beetles (Schedl 1957, 1958) revealed no particular ecological differences from *Afromicracis*.

The origin of Micracidini and its biogeographical history

Contrary to Wood's (1986) hypothesis, which implies repeated divergence between the African and American continents, our data revealed only a single origin of New World Micracidini. Most analyses indicated a nested position of this clade in Micracidini, implying an Afrotropical origin for the tribe as a whole. The only other scolytine clade with similar biogeographical pattern is the genus *Phrixosoma* Blandford (see Jordal and Cognato 2012). Although only four species of that genus were analysed genetically, it seems likely that the trans-Atlantic split in *Phrixosoma* reflects reciprocal monophyly and hence uncertainty in the geographical origin of that genus. Three clades in the weevil subfamily Platypodinae (Curculionidae) on the other hand indicate an African origin of Neotropical taxa (Jordal 2015), which is more in line with the pattern here detected for Micracidini. The age of divergence varies considerably between the three platypodine cases so there is apparently no common pattern underlying their evolutionary history. The colonisation of the New World in Micracidini occurred about the same time (late Cretaceous) as in the oldest trans-Atlantic split between the platypodine genera *Periommatus* Chapuis and *Tesserocerus* Saunders plus *Tesserocranulus* Schedl. That only a single ancestral population of micracidines got

permanently established in the New World over such a long time span is therefore particularly illustrative for their limited dispersal capacity.

Assessment of the micracidine sister group would further illuminate the origin of the tribe. Based on genetic data (see also Jordal and Cognato 2012) and our new morphological analysis, the most likely sister lineage to Micracidini is the tribe Hypoborini – here represented by the genus *Hypoborus*. Other potential sister groups tested in this study, such as *Cactopinus* and *Dendrochilus,* were nested within the tribe and as such were of little guidance in these questions. A complete sampling of all Micracidini genera will likely not influence much on the sister group assessment because they are all very similar to our included taxa. *Traglostus* is possibly a synonym of *Lanurgus* (Wood 1986) in which several species has recently been synonymised (Beaver 2011); *Saurotocis* is a derived form of *Pseudomicracis* (B.H.J., unpublished molecular data); *Stenoclyptus* may be a synonym of *Pseudothysanoes* (Wood 1986). The East African *Phloeocurus* is typical Afrotropical micracidine, intermediate between *Pseudomicracis and Lanurgus* in the shape of the antennae and declivity outline. The least known genus is the monotypic *Stevewoodia*, but it has all defining characters of *Afromicracis* and therefore could be an Afrotropical species introduced to the Caribbean area. Although we cannot yet conclude on the Micracidini sister relationship, it makes sense that a clade of dry adapted hypoborine beetles from mainly the Mediterranean and the arid parts of the Afrotropical and Malagasy regions (Jordal *et al*. 2004) forms the sister group to the dry adapted micracidine clade. Surely it will demand more molecular data to confirm this relationship, and we are currently optimising 13 additional genes for this purpose.

The application of morphological characters in Scolytinae phylogeny

This study is the first to include a large number of morphological characters in a phylogenetic analysis of a large number of scolytine genera. Although some incongruence was apparent between molecular and morphological data, the agreement between them was generally very high. In fact, the addition of 88 morphological characters to the molecular matrix resulted in substantially higher node support for many congruent nodes and as such document great value of including even a modest number of morphological characters in phylogeny reconstruction (Wiens 2003). When comparing nodes that were incongruent between the two data sets, none obtained high node support, which further emphasise the low conflict between these data sets. That previous classifications (Hopkins 1915; Wood 1978, 1986; Bright 2014) conflict strongly with our analyses suggest that these classifications were based on either insufficient data, or a total absence of proper character evaluation in a phylogenetic context.

At least some characters for each body part contributed to the phylogenetic resolution and there was no apparent difference in the information potential between internal and external characters. Even the most homoplasious groups of characters – such as those coded from flight wings and elytra – were at least partly diagnostic for smaller clades. One such example is the consistent presence of locking nodules along the internal rim of the elytra (just behind the scutellum) in Micracidini and *Cactopinus* while at the same time this character varied considerably in Hylurgini and Hylesinini where it is supposed to be invariably present (see Wood 1978). Internal or otherwise hidden characters from the mouthparts, proventriculus, or male genitalia, were particularly informative for Micracidini and close relatives and has previously demonstrated great use in resolving other scolytine tribes such as Dryocoetini and Xyleborini (Jordal *et al*. 2002a), Crypturgini (Jordal and Hewitt 2004), and in Polygraphini (Jordal 2009). The dissection of internal structures and membranes also resulted in some surprising discoveries. For instance, the suture separating the postnotum from the metanotum is presumably complete in all genera that Wood (1978, 1986) classified as "Scolytinae" as well as in some "Hylesininae", but we nevertheless found a fused postnotum in several genera of Cryphalini, but not in *Cryphalus*. The postnotum was also fused to the metanotum in Scolytoplatypodini (*Remansus* Jordal and *Scolytoplatypus* Schaufuss) and Hexacolini (*Gymnochilus* Eichhoff and *Scolytodes* Ferrari) – again reflecting the molecular data. Without going into further details here, it is clear that a thorough study of scolytine morphology is needed to correct apparent errors in character assessment

that have led to mistakes in classification of genera and tribes. This will be a topic for future studies that involves morphological dissections and DNA sequencing of almost 200 scolytine genera.

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Supplementary material

Appendices describing morphological characters and nexus file are available online at http://dx.doi.org/10.4039/tce.2016.31.

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